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Attorney Docket No.: 15966-557 CIP2 (CURA-57 CIP2)



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| APPLICANTS: | Jeffers et al. | | |
| SERIAL NUMBER: | 09/817,814 | EXAMINER: | Not yet assigned |
| FILING DATE: | March 26, 2001 | ART UNIT: | Not yet assigned |
| FOR: | Novel Fibroblast Growth Factor and Nucleic Acids Encoding Same | | |

**Box SEQUENCE**
Assistant Commissioner for Patents
Washington, D.C. 20231

**RESPONSE TO NOTICE TO FILE MESSING PARTS AND PRELIMINARY AMENDMENT**

This filing is in Response to the June 7, 2001 Notice of Missing Parts. Please amend the application as set forth below and consider the following remarks:

***In the Specification:***

Replace the paragraph starting on page 7, line 8 with:

--Figure 5 is a ClustalW alignment of FGF-CX with three other FGF family members. FGF-CX (SEQ ID NO:2) was aligned with human FGF-9 (SEQ ID NO:12), human FGF-16 (SEQ ID NO:9) and Xenopus FGF-CX (SEQ ID NO:24) (Accession Numbers D14838, AB009391 and AB012615, respectively).--

Replace the paragraph starting on page 7, line 26 with:

-- Figure 13 presents an analysis of the FGF-CX gene (SEQ ID NO:25), including the nucleotide and deduced amino acid sequence (SEQ ID NO:2) of FGF-CX. The initiation and stop codons are in bold, and an in frame stop codon residing in the 5' UTR is underlined.--

Replace the paragraph starting on page 107, line 2 with:

--E. coli strain BL21 (DE3) (Invitrogen) harboring the plasmid pET24a- FGF20X-del54-codon were grown in LB medium at 37°C. This plasmid encodes the C-terminal portion of FGF-CX beginning at position 55. When cell densities reached an OD of 0.6, IPTG was added to final

concentration of 1mM. Induced cultures were then incubated for an additional 4 hours at 37°C. Cells were harvested by centrifugation at 3000Xg for 15 minutes at 4°C, suspended in PBS and then disrupted with two passes through a microfluidizer. To separate soluble and insoluble proteins, the lysate was subjected to centrifugation at 10,000Xg for 20 minutes at 4°C. The insoluble fraction (pellet) was extracted with PBS containing 1M L-arginine. The remaining insoluble material was then removed by centrifugation and the soluble fraction of the arginine extract was filtered through 0.2 micron low-protein binding membrane and analyzed by SDS PAGE. The result is shown in Fig. 25, which indicates that the product is a polypeptide with an apparent molecular weight of approximately 20 kDa (see arrow). N-terminal sequencing of the expressed polypeptide provides the sequence AQLAHLHGILRRRQL which is 100% identical to residues 55-69 of FGF-CX (Fig. 1, SEQ ID NO:2).--

***In the claims:***

Replace claims 63 and 64 with amended claims 63 and 64 below:

63. The polypeptide fragment described in claim 2, wherein the fragment comprises an amino acid sequence selected from the group consisting of residues 55-211 of SEQ ID NO:2 and residues 24-211 of SEQ ID NO:2.

64. The isolated nucleic acid molecule described in claim 6, wherein the nucleic acid molecule comprises a nucleic acid sequence encoding a polypeptide fragment comprising an amino acid sequence selected from the group consisting of residues 55-211 of SEQ ID NO:2 and residues 24-211 of SEQ ID NO:2.

Insert the submitted sequence listing pages 1-13, at the end of the specification.

**REMARKS**

The specification has been amended in response to the June 7, 2001 Notice to File Missing Parts. Applicants submit herewith a computer readable form (CFR) copy of the "Sequence Listing," an initial paper copy of the "Sequence Listing," and a statement that the content of the paper and computer readable copies are the same and include no new matter, in

compliance with 37 C.F.R. §§ 1.821-1.825. The specification has been amended to insert the sequence listing.

In the original application, SEQ ID NOs were not applied to the nucleotide or amino acid sequences in Figures 5 and 13. These sequences have been assigned these SEQ ID NOs to match with the sequence listing. Also amendments are made to correct an error in the enumeration of amino acids provided in the specification and claims concerning the deletion variant disclosed in Example 16. That the sequence AQLAHLHGILRRRQL given in Example 16 is at position 55-69 of SEQ ID NO:2 would be obvious to a person skilled in the art when making a simple comparison between these sequences. Since the alignment of the determined N terminal sequence with the full length sequence provides the appropriate amino acid residue numbers, Applicants state that no new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

The Commissioner is authorized to charge any fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Attorney Reference No. 15966-557 CIP2 (Cura-57 CIP2).

Respectfully submitted,

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**Version with markings to show changes made**

Amend the paragraph starting on page 7, line 8 as follows:

Figure 5 is a ClustalW alignment of FGF-CX with three other FGF family members. FGF-CX (SEQ ID NO:2) was aligned with human FGF-9 (SEQ ID NO:12), human FGF-16 (SEQ ID NO:9) and Xenopus FGF-CX (SEQ ID NO:24) (Accession Numbers D14838, AB009391 and AB012615, respectively).

Amend the paragraph starting on page 7, line 26 as follows:

Figure 13 presents an analysis of the FGF-CX gene (SEQ ID NO:25), including the nucleotide and deduced amino acid sequence (SEQ ID NO:2) of FGF-CX. The initiation and stop codons are in bold, and an in frame stop codon residing in the 5' UTR is underlined.

Amend the paragraph starting on page 107, line 2 as follows:E. coli strain BL21 (DE3) (Invitrogen) harboring the plasmid pET24a- FGF20X-del54-codon were grown in LB medium at 37°C. This plasmid encodes the C-terminal portion of FGF-CX beginning at position 55. When cell densities reached an OD of 0.6, IPTG was added to final concentration of 1mM. Induced cultures were then incubated for an additional 4 hours at 37°C. Cells were harvested by centrifugation at 3000Xg for 15 minutes at 4°C, suspended in PBS and then disrupted with two passes through a microfluidizer. To separate soluble and insoluble proteins, the lysate was subjected to centrifugation at 10,000Xg for 20 minutes at 4°C. The insoluble fraction (pellet) was extracted with PBS containing 1M L-arginine. The remaining insoluble material was then removed by centrifugation and the soluble fraction of the arginine extract was filtered through 0.2 micron low-protein binding membrane and analyzed by SDS PAGE. The result is shown in Fig. 25, which indicates that the product is a polypeptide with an apparent molecular weight of approximately 20 kDa (see arrow). N-terminal sequencing of the expressed polypeptide provides the sequence AQLAHLHGILRRRQL which is 100% identical to residues 5[4]5-6[4]9 of FGF-CX (Fig. 1, SEQ ID NO:2).

Amend claims 63 and 64 as follows:

63. The polypeptide fragment described in claim 2, wherein the fragment comprises an amino acid sequence selected from the group consisting of residues [54] 55-211 of SEQ ID NO:2 and residues 24-211 of SEQ ID NO:2.

64. The isolated nucleic acid molecule described in claim 6, wherein the nucleic acid molecule comprises a nucleic acid sequence encoding a polypeptide fragment comprising an amino acid sequence selected from the group consisting of residues [54] 55-211 of SEQ ID NO:2 and residues 24-211 of SEQ ID NO:2.